# Protein kinases coordinately regulate the p53

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# Sentinels of DNA integrity in stem cells

## Safeguarding future generations of cells

Integrated into the somatic cell cycle are multifaceted mechanisms to protect genomic fidelity from genotoxic threats occurring during cell division or cellular quiescence. How embryonic stem cells respond to an array of attacks on genomic integrity has been uncertain, particularly in light of embryonic-like rapid cell cycle phases versus adult cells and the lack of an effective $G_1$/S checkpoint. Whether a DNA damage response is activated similarly to somatic cells or apoptotic pathways used to purge damaged cells are important questions, since the longevity of embryonic stem cells provides opportunities for accumulated mutations and a source for carcinogenic cells. In this issue, Chuyikin et al. investigate the timing and sensitivity of the DNA damage response pathway to double strand breaks (DSBs) in mouse embryonic stem cells (ESCs), validating its responsiveness and providing a comprehensive view of key signaling events.

DNA DSBs are potently mutagenic lesions incurring chromosome breaks, potential rearrangements, mutation and loss of information.[1] The cellular response is immediate, sensitive and persistent, occurring within 30 seconds of damage upon detection of as little as 8 DSBs per cell. The response can be fully active in 15 minutes and persist for hours. Repair is preferred and may elicit checkpoint delays to cell cycle progression, with extreme genotoxic conditions initiating apoptotic pathways. The majority of DSB proteins are activated by PI-3 like kinases, with the primary mammalian response to DSBs occurring via the ATM kinase that is able to respond directly to DSBs. Phosphorylated downstream targets include the uncommon histone, H2AX. This histone provides a cytological platform at DSB sites for the recruitment of DSB mediator and effector proteins such as MDC1 and NBS1. To this scaffold further DSB proteins are recruited, amplifying the signal. NBS1 is part of the MRN complex that includes MRE11 and Rad50 and mediates nuclear localization of the complex to the DNA for stabilizing chromatin ends. The nucleolytic processing of DNA ends by MRE11 resection triggers a second pathway modulated by ATR, that responds to RPA coated ssDNA. Chuyikin et al., used antibodies to phosphorylated ATM and H2AX (pATM, pH2AX) as sensitive temporal markers of DNA repair foci that form at DSBs and followed these events through the cell cycle.

In fast proliferating undifferentiated cells an increase in single strand DNA breaks (SSBs) is typically observed, attributed to ongoing DNA replication, and not generally considered mutagenic. Chuyikin et al. used sensitive comet assays along with pH2AX and pATM antibodies to confirm the presence of SSBs in mESCs and a low background of pH2AX positive/pATM absent poised foci. Upon γ-irradiation to induce DSBs, dramatic detection of DNA repair foci including both pH2AX and pATM occurs. FACs analysis indicated no cell cycle arrest at $G_1$/S from γ-irradiation, although a slight delay at $G_2$/M. Chuyikin et al. did find that mESCs have an active spindle assembly checkpoint allowing cells to be blocked at $G_2$/M with nocodazole and then released synchronously through the cell cycle. The key to their detection of this checkpoint was a six hour treatment with drug, versus longer timepoints. Indeed Reider and Maiato[2] have shown that in mammalian cells, spindle assembly checkpoint duration is variable and need not be satisfied to be overridden by adaptation, slippage or leakage, quite unlike the tight cell cycle arrest observed in fungi. Therefore longer treatments with nocodazole to arrest mESCs at this stage would be expected to simply be ineffective and promote further polyploidy by attenuating the mitotic mechanism. The authors detailed analysis of induction of DNA repair foci in all cell cycle stages revealed that all stages generate foci, including metaphase chromosomes in mitosis, although foci were most prominent in $G_1$, $G_2$ phases. Thus the primary response by the ATM pathway in these cells is not limited by cell cycle phase.

The maintenance of genomic fidelity in ESCs may require more enhanced DNA repair[3] as well as alternative mechanisms to DNA repair, such as increased apoptosis. Chuyikin et al. observed increased caspase activity triggered after γ-irradiation of mESCs, but found no significant increase in cell death. They also found that protein levels of p53, a downstream target of the ATM kinase that is important for the $G_1$/S checkpoint as well as p53-dependent apoptosis, were comparable to fibroblast cells, however p53 lacked activating phosphorylation. Both of these observations help to explain an ineffective $G_1$/S checkpoint and the need for p53-independent apoptosis.

Additional alternate mechanisms for maintaining genomic integrity ESCs have been reported and contribute. This includes a 100X reduction in mutations versus somatic cells and resistance to oxidative stress. Asymmetry mechanisms,[4] that are a commonly used means of cellular signaling and polarity from yeast to man may also apply, as in the Cairns immortal strand hypothesis. In 1975 Cairns proposed that stem cells might minimize mutations to their genomes from DNA replication by asymmetric segregation of their DNA. Retention of parental strands in the stem cell and segregation of potential mutation carrying DNAs into non-stem cell or differentiating daughters could reduce the mutation potential.[4] Such asymmetric sister chromatid strand segregation is still controversial despite having been observed during mitosis in several stem cell populations. Continued elegant studies, such at that by Chuyikin et al., that define which pathways are present and examine the crosstalk in pathways used to detect, signal, repair and protect genomic integrity will continue to provide exciting new systemic views into stem cells. Our therapeutic use of stem cells in the future including understanding of cellular differentiation and cancer depends on it.

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Janet L. Paluh; Rensselaer Polytechnic Institute; Troy, New York USA; Email: Paluhj@rpi.edu

# Protein kinases coordinately regulate the p53

## ΔNp63α axis after DNA damage

The ancestral member of the *p53* gene family, *p63*, is overexpressed in head and neck squamous cell carcinomas (HNSCC). To date little is known about the regulation of the N-terminal truncated isoform of p63 (ΔNp63α) which is considered a marker of certain epithelial stem cells and cancer stem cells. In this issue of *Cell Cycle*, a study provides insight into the DNA-damage induced post-translational regulation of ΔNp63α protein stability by the ATM pathway. The data imply that ATM has evolved to act as a master regulator in the basal skin stem cell to co-ordinately switch off ΔNp63α and switch on p53 after irradiation. This would attenuate the proliferative capacity of the stem cell in order for DNA damage to be repaired by the p53 response. These data also have implications for how inactivation of the ATM pathway could promote carcinogenesis in squamous cancers via upregulation of ΔNp63α and for developing novel therapeutic strategies for inhibiting ΔNp63α in cancer stem cell populations.

*Protein kinase control of p53: Phosphorylation activates p53*. Regulation of the p53 tumor suppressor protein involves sets of post-translational modifications, including phosphorylation, acetylation, and ubiquitination, which mediate p53 protein stability and activational status.[1] Biochemical and genetic studies have together solved the functions of some p53 kinase pathways.[2] For example, ATM is the pre-dominating genetic activating factor for p53, but how

ATM phosphorylation of p53 alters its function biochemically is not so well-defined. By contrast, phosphorylation of the C-terminal CK2-site of p53 stabilizes p53:DNA interactions, phosphorylation of the CK2 site on p53 is induced upon irradiation, and alanine substituted CK2-site p53 transgenic mice have elevated damage-induced bladder cancer and UV-induced skin cancer.[3] Clinical studies have subsequently shown that the UV-induced phosphorylation of p53 at the CK2 site is confined to the basal or stem-cell compartment in human skin rather than the suprabasal layer where p53 protein is predominantly stabilized after DNA damage.[4] These data highlight the cell-specificity in kinase catalyzed activation of the p53 tumor suppressor function and also the intriguing possibility that skin stem cell populations have evolved the recruitment of specific kinase pathways to maintain stem cell genome integrity.

*Protein kinase control of ΔNp63: Phosphorylation destabilizes ΔNp63.* The *p53* gene has evolved from an ancestral mother gene named *p63*.[5] The structure of the *p63* gene is very similar to that of *p53*, consisting of an N-terminal transactivation (TA) domain, a highly conserved core DNA-binding domain and a C-terminal oligomerization domain. The *p63* gene contains an internal promoter leading to the expression of a transcriptionally active (TA) isoform and an N-terminally truncated (ΔN) protein isoform named ΔNp63α, which can function as a transcription factor in its own rite. *p63* knockout mice have severe developmental defects, including abnormal development of limbs, stratified epidermis and epidermal tissues, and die shortly after birth.[5] Particularly relevant to the phosphorylation of p53 in damaged skin stem cells, the expression of ΔNp63α is restricted to the basal compartment of the stratified epithelia, suggesting a role in maintaining the proliferative compartment.[6]

The expression of these two p53 family members in basal cell populations raises the question of whether regulation of the pro-proliferative ΔNp63α and anti-proliferative p53 protein pathways are co-ordinated after DNA damage; i.e. ΔNp63α protein is de-stabilized after DNA damage using in vitro and in vivo systems[7,4] whilst upregulation of p53 phosphorylation occurs at the CK2 and ATM sites in basal skin cells in vivo.[4] Is there a kinase pathway that could both switch ΔNp63α off and p53 on when the stem cells need to maintain genome stability after DNA damage? A paper in this issue of *Cell Cycle* (Haung et al., 2008) addresses this question revealing that ΔNp63α is sequentially phosphorylated after DNA damage by ATM, CDK2 and p70s6K. This DNA damage induced phosphorylation of ΔNp63α leads to a dramatic decrease in ΔNp63αprotein levels in HNSCC cells, which is abated in the absence of these protein kinases.[8] Although the biochemical effects of each phosphorylation on ΔNp63α protein degradation is not defined, these data provide a co-ordinated mechanism for switching ΔNp63α off and p53 on involving the known p53 activator, ATM, as the master switch in this axis.[9] Similarly, as CDK2 has been implicated in stimulating p53 function after irradiation,[15] this kinase pathway might also coordinate p53 activation and ΔNp63α protein degradation. These data together suggest that at least two protein kinase pathways play dual roles in controlling p53 family function after DNA damage.

*Implications of ATM regulation of ΔNp63α in cancer.* There is increasing evidence that cancer is a stem cell disease. Adult stem cells are long lived cells that are able to accumulate the number of genetic mutations necessary to result in tumor formation. It is conceivable that overexpression of genes which maintain stem cells, and subsequently cancer stem cells, with a proliferative advantage would be beneficial to the establishment and progression of cancer. Studies indicate that ΔNp63α is specifically expressed in epidermal stem cells possessing the highest proliferative capacity, and that ΔNp63α expression is lost as these cells migrate from the basal layer and become terminally differentiated cells.[10] Subsequently, p63 has been shown to be essential for maintaining the proliferative capacity of epidermal stem cells and preventing premature entry into terminal differentiation.[10] Parallel and p63-independent functions are carried out in other stem cell compartments, for example Bmi-1[11] and Plzf[12] are essential for maintaining the proliferative potential of hematopoietic and spermatogenic stem cells, respectively. Hence, maintaining a state of high proliferative potential appears to be a common strategy to avert differentiation and enable extensive programmes of cell division. Therefore overexpression of these factors which maintain proliferation and prevent differentiation may be of selective advantage to cancer stem cells.

Head and neck squamous cell carcinomas are malignancies derived from cells within the basal epithelia of the aerodigestive mucosa and are usually treated with a combination of irradiation and chemotherapy.[13] The majority of head and neck tumors harbour inactivating mutations affecting the *p53* gene[13] indicating that selection pressures have been placed on p53 pathway silencing. However, the most ancient member of the *p53* gene family, *p63*, has also been implicated in squamous cancers.[13] Although the primary function of *p63* gene family function is in ectodermal differentiation during development and stratified epithelial progenitor cell maintenance, the over-expression of ΔNp63α is observed in numerous squamous cell carcinomas, including head and neck, lung, prostate, bladder and breast, suggesting that *p63* can act as an oncogene.[5] In fact, ΔNp63 can transcriptionally up-regulate proteins implicated in pro-survival pathways including Heat shock protein 70 (hsp70), Multi Drug Resistant gene 1 (MDR1), the epidermal growth factor receptor (EGFR), and the integrin family,[14] providing a mechanism for the pro-proliferative functions of this isoform in stem cells and in cancers. As this issue of Cell Cycle has illustrated that specific post-translational modifications of ΔNp63α via ATM and related kinase activities are associated with ΔNp63α degradation, it is possible that the enhanced production of ΔNp63α in cancer stem cells occurs via a defect in protein kinase-mediated degradation of ΔNp63α. Accordingly, strategies that promote, for example, ATM-mediated degradation of ΔNp63α in cancer stem cells may yield novel therapeutics that enable us to directly target the root of squamous cell carcinomas.

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Lisa Y. Pang; The University of Edinburgh Hospital for Small Animals; Edinburgh, Scotland UK; Ted R. Hupp; The University of Edinburgh; Cancer Research UK; Edinburgh, Scotland UK; Email: ted.hupp@ed.ac.uk